gibco

ExpiSf™ Expression System

USER GUIDE

For superior baculovirus-based protein expression using high-density ExpiSf9™ cells in a chemically defined, yeastolate-free medium

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Revision	Date	Description
A.0	26 April 2018	New document

Contents

Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Gibco™ ExpiSf™ Expression System is a high-yield baculovirus-based insect expression system based on suspension-adapted Sf9 (*Spodoptera frugiperda*) cells. The ExpiSf™ Expression System Starter Kit provides cells, culture medium, and reagents to infect 1 liter of cell culture.

Contents and storage

The ExpiSf™ Expression System contains the following components. For a detailed description see "Components of ExpiSf™ Expression System".

Contents[1]	Amount	Storage
ExpiSf9™ Cells (1 × 10^7 cell/mL)	2 × 1.5 mL	Liquid nitrogen, vapor phase[2]
ExpiSf™ Protein Production Kit: • ExpiSf™ CD Medium • ExpiSf™ Enhancer	— 1 L 4 × 1 mL	2°C to 8°C. Protect from light.
ExpiFectamine™ Sf Transfection Reagent	1 mL	2°C to 8°C. Do not freeze.
Opti-MEM™ I Reduced Serum Medium [3]	500 mL	2°C to 8°C. Protect from light.
pFastBac™ 1 Expression Vector (0.5 µg/ µL) [3,4]	10 µg	–20°C
pFastBac™ 1-Gus Control Vector (0.2 ng/ µL) ([3,4]	4 ng	–20°C
MAX Efficiency™ DH10Bac™ Competent Cells[3]	5 × 100 µL	Competent cells: –80°C pUC19 DNA: –20°C SOC Medium: 4°C or room temperature
Nalgene™Single-Use PETG 125-mL non-baffled, vented Erlenmeyer Flasks[5]	24	Room temperature

[1] Some components are not available in all regions.
[2] Store the frozen cells in liquid nitrogen until ready to use. Do not store the cells at –80°C.
[3] Product not included in Cat. No. A39111
[4] In TE buffer, pH 8.0: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0
[5] Product not available in all global regions.

Components of ExpiSf™ Expression System

ExpiSf™ Expression System

The ExpiSf™ Expression System is designed to allow high-density infection of suspension ExpiSf9™ cells in a chemically defined, yeastolate-free, serum-free, protein-free, animal origin-free medium. The system includes ExpiSf9™ cells that have been adapted to serum-free, high-density suspension culture in ExpiSf™ CD Medium. Transfection, baculovirus production, and recombinant protein expression experiments can be performed directly in ExpiSf™ CD Medium. The ExpiSf™ Expression System Starter Kit provides cells, culture medium, and reagents to infect 1 liter of cell culture. The kit also includes the pFastBac™ 1 Expression Vector and MAX Efficiency™ DH10Bac™ Competent Cells for generation of bacmid DNA using the Bac-to-Bac™ Baculovirus Expression System. Except for the MAX Efficiency™ DH10Bac™ Competent Cells, all the components of the ExpiSf™ Expression System are animal origin-free.

ExpiSf9™ cells

The ExpiSf9™ cell line is a derivative of the Gibco™ Sf9 cell line. ExpiSf9™ cells are adapted to high-density suspension culture in ExpiSf™ CD Medium. Frozen cells are supplied in, and can be thawed directly into, ExpiSf™ CD Medium. ExpiSf9™ cells exhibit the following characteristics:

- Derived from the same parental lineage as the Gibco™ Sf9 cell line
- Rapid recovery post-thaw
- Adapted to high-density, serum-free, suspension growth in the chemically defined, yeastolate-free ExpiSf™ CD Medium
- Doubling time of approximately 24 hours during log phase growth
- Maximum cell densities of approximately 20×10^6 cells/mL in shake flask culture
- High baculovirus production and protein expression
- Stable growth and expression over multiple passages

Note: ExpiSf9™ cells are also available separately, see Appendix H, "Ordering information".

ExpiSf™ CD Medium

ExpiSf™ CD Medium is a chemically defined, yeastolate-free medium developed specifically for the high-density culture, transfection, and infection of ExpiSf9™ cells in suspension.

ExpiSf™ CD Medium exhibits the following features:

- Formulated as an optimized, chemically defined, yeastolate-free, serum-free, protein-free, animal origin-free medium to support the high-density culture, transfection, and baculovirus infection of ExpiSf9™ cells in suspension.
- Ready-to-use format with no additional supplementation required.
- Does not interfere with nor reduce the activity of ExpiFectamine™ Sf Transfection Reagent.
- Designed for scalable transfection, baculovirus production, and protein expression.

Note: The ExpiSf™ CD Medium is also available separately, see Appendix H, "Ordering information".

ExpiFectamine™ Sf Transfection Reagent

ExpiFectamine™ Sf Transfection Reagent is optimized for the transfection of nucleic acids into high-density ExpiSf9™ cultures.

ExpiFectamine™ Sf Transfection Reagent exhibits the following features:

- Enables efficient transfection of bacmid DNA in suspension ExpiSf9™ cells in ExpiSf™ CD Medium using an improved, faster complexation protocol.
- Supports transfection in both adherent and suspension ExpiSf9™ cultures.
- Low toxicity cationic lipid-based reagent. ExpiFectamine™ Sf/bacmid DNA complexes can be added directly to cells in ExpiSf™ CD Medium and it is not necessary to change or add medium following transfection.

ExpiSf™ Enhancer

ExpiSf™ Enhancer is a proprietary, chemically defined, animal origin-free formulation developed to be used along with ExpiSf™ CD Medium to enhance protein production in ExpiSf9™ cells, resulting in maximal protein yields.

Note: ExpiSf™ Enhancer is a component of the ExpiSf™ Protein Production Kit. The ExpiSf™ Protein Production Kit is available separately in multiple sizes, see Appendix H, "Ordering information".

Opti-MEM™ I Reduced Serum Medium

Opti-MEM™ I Reduced Serum Medium is a serum-free, chemically defined, low protein medium used to complex bacmid DNA with ExpiFectamine™ Sf Transfection Reagent, providing optimal lipid-DNA complex formation and efficient transfection. Opti-MEM™ I Reduced Serum Medium is also available separately, see Appendix H, "Ordering information".

Bac-to-Bac™ Baculovirus Expression System components

The ExpiSf™ Expression System has been optimized to work in concert with the Bac-to-Bac™ Baculovirus Expression System for rapid and efficient generation of recombinant baculoviruses (for more information on the Bac-to-Bac™ Baculovirus Expression System, visit **thermofisher.com/bactobac**. The two major components of the Bac-to-Bac™ Baculovirus Expression System, pFastBac™ 1 Expression Vector and MAX Efficiency™ DH10Bac™ Competent Cells, as well as pFastBac™ 1-Gus Control Vector are conveniently included in the ExpiSf™ Expression System Starter Kit for expression of your gene of interest in ExpiSf9™ cells.

pFastBac™ 1 Expression Vector

pFastBac™ 1 Expression Vector is a non-fusion donor plasmid that is used to clone your gene of interest using restriction enzyme digestion and ligation. Gene expression is controlled by the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) polyhedrin (PH) promoter for high-level baculovirus-mediated protein expression in ExpiSf9™ cells. For more information on using the pFastBac™ 1 Expression Vector, see "Generate pFastBac™ construct" on page 29.

pFastBac™ 1 Expression Vector is also available separately, see Appendix H, "Ordering information".

MAX Efficiency™ DH10Bac™ Competent Cells

MAX Efficiency™ DH10Bac™ Competent Cells contain a baculovirus shuttle vector (bacmid) with a mini-attTn7 target site and a helper plasmid, and allows generation of a recombinant bacmid following transposition of the pFastBac™ expression construct. Once the pFastBac™ 1 donor plasmid containing the gene of interest is transformed into MAX Efficiency™ DH10Bac™ Competent Cells, transposition occurs between the mini-Tn7 element on the pFastBac™ vector and the mini-attTn7 target site on the bacmid to generate a recombinant bacmid. This transposition reaction occurs in the presence of transposition proteins that are supplied by the helper plasmid.

MAX Efficiency™ DH10Bac™ Competent Cells are also available separately, see Appendix H, "Ordering information".

pFastBac™ 1-Gus Control Vector

pFastBac™ 1-Gus Control Vector is provided as a positive control for baculovirus production and protein expression using the ExpiSf™ Expression System. The vector contains the *Arabidopsis thaliana* gene for β-glucuronidase (Gus) and can be used to produce recombinant baculovirus, which, when used to infect ExpiSf9™ cells, express β-glucuronidase enzyme. The Gus protein that is produced in ExpiSf9™ cells after infection with Gus-expressing baculovirus is present in the intracellular fraction, with the optimal yields obtained in three days. For more information on quantifying Gus protein, see "Assay for β-glucuronidase" on page 37.

pFastBac™ 1-Gus Control Vector is also available as part of multiple Bac-to-Bac™ expression vector products, see Appendix H, "Ordering information".

Methods

Guidelines for ExpiSf9™ cell culture

Cell handling

- All solutions and equipment that come in contact with the cells must be sterile. Always use proper sterile technique and work in a laminar flow hood.
- On receipt, grow and freeze multiple vials of ExpiSf9™ cells to ensure that you have a sufficient supply of early-passage cells.

  Note: Store the frozen cells in liquid nitrogen, vapor phase until ready to use. Do not store the cells at −80°C.
- Avoid short-term, extreme temperature changes. When storing cells in liquid nitrogen following receipt on dry ice, allow the cells to remain in liquid nitrogen for 3–4 days before thaw.
- For cell manipulations, mix the cells by gentle swirling. Avoid vigorous shaking or pipetting.
- Before starting experiments, ensure that the cells are established by allowing freshly thawed cells to recover in culture for two or more passages post-thaw before transfection or infection.
- ExpiSf9™ is a robust cell line that is adapted to high-density, suspension growth and has a doubling time of approximately 24 hours during log phase growth. The cells have a broad log-phase growth window of 4×10^6–12×10^6 cells/mL, with a maximum density of approximately 20×10^6 cells/mL in shake flask cultures.
- Passage ExpiSf9™ cells when they reach a density of 5×10^6–10×10^6 viable cells/mL (i.e., early to mid-log-phase of growth), typically every 3–4 days.

  Note: Do not subculture cells until they reach a density of at least 5×10^6 viable cells/mL. Cells that are subcultured at lower densities can show longer doubling times and lower titers over time. Modify seeding density to attain the target cell density of at least 5×10^6 viable cells/mL at the time of subculturing.
- Use a hemocytometer with the trypan blue exclusion method, or an automated cell counter, to determine cell viability. Ensure log phase cultures are ≥90% viable.
- Transfer cells into pre-warmed room temperature medium when thawing or subculturing cells.

Growth media

IMPORTANT! ExpiSf™ CD Medium is sensitive to light. For optimal results, use and store media protected from light.

- ExpiSf™ CD Medium is formulated in a ready-to-use format.
- For suspension growth and expression applications, use ExpiSf™ CD Medium without any supplementation.

Thaw and establish ExpiSf9™ cells

General guidelines

- ExpiSf9™ cells are supplied in a vial containing 1.5 mL of cells at 1×10^7 viable cells/mL in 92.5% ExpiSf™ CD Medium and 7.5% DMSO
- Thaw the cells directly into ExpiSf™ CD Medium, pre-warmed to room temperature

Required materials not supplied

See Appendix H, "Ordering information".

- Hemocytometer or an automated cell counter
- Trypan Blue Solution, 0.4%
- Orbital shaker in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator (See Table 2 for recommended shaker speeds)

Thaw ExpiSf9™ cells

1. Remove the vial of cells from liquid nitrogen and swirl in a 37°C water bath for 1 to 2 minutes to thaw the cells rapidly until only a small amount of ice remains.

   Do not submerge the vial in the water.

2. Just before the cells are completely thawed, decontaminate the vial by wiping it with 70% ethanol before opening it in a laminar flow hood.

3. Use a 2-mL or 5-mL pipette, to transfer the entire contents of the cryovial into a 125-mL PETG, sterile, non-baffled, vented Erlenmeyer shake flask containing 25 mL of pre-warmed ExpiSf™ CD Medium.

4. Incubate cells in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator on an orbital shaker platform.

   Note: Set the shake speed to 125 ±5 rpm for shakers with a 19-mm or 25-mm shaking diameter, or 95 ±5 rpm for shakers with a 50-mm shaking diameter.

5. Three days post-thaw, determine viable cell density and percent viability.

   Cell viability should be ≥80% by three days post-thaw.

6. Continue to monitor cell density and viability, then subculture the cells once the culture has reached 5×10^6–10×10^6 viable cells/mL (typically 4–5 days post-thaw), see "Passage ExpiSf9™ cells" on page 13.

Subculture ExpiSf9™ cells

General guidelines

- ExpiSf9™ cells are capable of achieving high cell densities; therefore, we recommend that the cells attain a density of 5×10^6–10×10^6 viable cells/mL at the time of subculturing.
- Cells may exhibit some clumping during routine cell culture maintenance when they reach higher densities.
- Clumping will not reduce performance of the cells for transfections and infections.

Required materials not supplied

- ExpiSf9™ cell cultures at 5×10^6–10×10^6 viable cells/mL
- Hemocytometer or an automated cell counter
- Trypan Blue Solution, 0.4%
- Orbital shaker in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator (see Table 2 for recommended shaker speeds)

Passage ExpiSf9™ cells

1. Use the viable cell density to calculate the volume of cell suspension required to seed a new shake flask according to the recommended seeding densities in Table 1 and the recommended culture volumes in Table 2.

Table 1 Recommended seeding densities for routine cell culture maintenance

Subculture timing	Recommended seeding density
For cells ready 3 days post-subculture	0.7×10^6–1.0×10^6 viable cells/mL
For cells ready 4 days post-subculture	0.4×10^6–0.6×10^6 viable cells/mL

Table 2 Recommended volumes and shake speed for routine cell culture maintenance in vented, non-baffled flasks

Flask size	Culture volume	Shake speed[1]
125 mL	25–30 mL	125 ± 5 rpm (19-mm shaking diameter) 125 ± 5 rpm (25-mm shaking diameter) 95 ± 5 rpm (50-mm shaking diameter)
250 mL	50–60 mL	
500 mL	100–120 mL	
1 L	200–240 mL	
2 L	400–480 mL	
3 L	600–800 mL	80 ± 5 rpm (19-mm shaking diameter)

[1] Use higher range of recommended shake speeds for larger culture volumes shown.

2. Transfer the calculated volume of cells to fresh, pre-warmed ExpiSf™ CD Medium in a shake flask.

3. Incubate the cells in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator on an orbital shaker platform until cultures reach a density of 5×10^6–10×10^6 viable cells/mL.

   Note: Cells that are subcultured at densities outside of this log-phase growth window may show longer doubling times and lower titers over time. Modify the initial seeding density to attain the target cell density of 5×10^6–10×10^6 viable cells/mL at the time of subculturing.

   Note: Refer to Table 2 for recommended shake speed for shakers with various shaking diameters.

4. Repeat Steps 1–3 to maintain or expand the cells for transfection or infection.

   See "Cryopreserve cells" for recommendations on freezing the cells.

Cryopreserve cells

1. Freeze ExpiSf9™ cells at a final density of 1×10^7 viable cells/mL in 1.5 mL total volume of 92.5% conditioned ExpiSf™ CD Medium and 7.5% DMSO.

2. Allow cells to attain a viable cell density of 3×10^6–4.5×10^6 cells/mL (typically 3 days post-passaging from a seeding density of 0.5×10^6–0.6×10^6 cells/mL) and ≥90% viability before harvest.

   Note: For cryopreservation, the viable cell density at time of harvest is critical for optimal cell health. Therefore, make sure to only harvest cells when they are within the recommended 3×10^6–4.5×10^6 viable cells/mL range. If viable cell density is too low at the time of harvest, return cells to the incubator for an extra day. If viable cell density is too high at the time of harvest, subculture the cells at 0.5×10^6–0.6×10^6 cells/mL and prepare for harvest again after 3–4 days.

3. Determine the required volume of conditioned ExpiSf™ CD Medium and DMSO according to the following table.

Component	Final concentration
Conditioned ExpiSf™ CD Medium	92.5%
Dimethyl Sulfoxide (DMSO)	7.5%

4. Centrifuge the cells at 300 × *g* for 5 minutes to pellet.

5. Decant the spent ("conditioned") medium into a sterile conical tube or bottle.

6. Gently resuspend the cell pellet from Step 4 using the appropriate volume of conditioned medium collected in Step 5 to achieve a final cell density of 1×10^7 viable cells/mL.

7. Add the required volume of DMSO to the cell suspension and gently mix.

8. Immediately aliquot 1.5 mL cell volume per cryovial.

9. Freeze the cells in an automated or manual controlled-rate freezing apparatus following standard procedures.

   For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.

10. Transfer frozen vials to liquid nitrogen, vapor phase for long-term storage.

Baculovirus generation: Transfect ExpiSf9™ cells

General guidelines

- Use ExpiFectamine™ Sf Transfection Reagent for optimal transfection of high-density suspension ExpiSf9™ cultures with bacmid DNA for generation of recombinant baculovirus.
- See "Generate pFastBac™ construct" on page 29 for detailed instructions to generate bacmid DNA for your gene(s) of interest using the Bac-to-Bac™ Baculovirus Expression System.
- ExpiFectamine™ Sf Transfection Reagent is a low toxicity reagent formulated to enable transfection without the need to change or add media post-transfection.

Required materials not supplied

See Appendix H, "Ordering information".

- ExpiSf9™ cells in ExpiSf™ CD Medium at 5 × 10^6–10 × 10^6 viable cells/mL and ≥90% viability.
- Bacmid DNA preparation, sterile, free from phenol and sodium chloride, and containing mostly supercoiled DNA

  Note: We recommend isolating bacmid DNA using the PureLink™ HiPure Plasmid Kit. Follow instructions in *PureLink™ HiPure Plasmid Purification Kits User Guide*, (Pub. No. MAN0000486) using the MaxiPrep low-copy plasmid instructions. See Appendix H, "Ordering information").
- Gus bacmid DNA Control generated using pFastBac™ 1-Gus Control Vector
- **Note:** Do not add antibiotics to media during transfection because it can decrease transfection efficiency.
- Orbital shaker in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator (see Table 2 for recommended shaker speeds)
- Hemocytometer or an automated cell counter
- Trypan Blue Solution, 0.4%

ExpiSf9™ cell transfection at different scales

Transfection of bacmid DNA using the ExpiSf™ Expression System is directly scalable from 24 deep-well plate to 500-mL shake flasks using suspension ExpiSf9™ culture format.

Table 3 Recommended volumes at different scales

The amounts provided are on a per-well or per-flask basis.

Vessel type	24 deep-well plate	125-mL	250-mL	500-mL
Total number of cells	10 × 10^6 cells	62.5 × 10^6 cells	125 x 10^6 cells	250 x 10^6 cells
Culture volume to transfect	4 mL	25 mL	50 mL	100 mL
Shake speed[1]	250 ± 5 rpm	125 ± 5 rpm (19 mm shaker diameter) 125 ± 5 rpm (25 mm shaker diameter) 95 ± 5 rpm (50 mm shaker diameter)		
Amount of plasmid DNA	1 µg	12.5 µg	25 µg	50 µg
ExpiFectamine™ Sf Transfection Reagent	5 µL	30 µL	60 µL	120 µL

Vessel type	24 deep-well plate	125-mL	250-mL	500-mL
Opti-MEM™ I Reduced Serum Medium[2]	250 µL	1 mL	2 mL	4 mL
Volume of bacmid DNA[3]	2–4 µL	25–50 µL	50–100 µL	100–200 µL
Recommended flask type	—	Non-baffled, vented shake flask		
Culture type	Suspension			

[1] Recommended shake speed ranges; optimal shake speed should be determined empirically based on the specific laboratory equipment used. Also see Appendix A, "Troubleshooting".

[2] Volume of Opti-MEM™ I Reduced Serum Medium used to dilute ExpiFectamine™ Sf Transfection Reagent

[3] Assuming a bacmid DNA stock concentration of 250 – 500 µg/mL

ExpiSf9™ cell transfection

- Allow freshly thawed cells to recover in culture for two or more passages post-thaw before transfecting.
- During all cell manipulations, mix the cells by gentle swirling; avoid vigorous mixing/pipetting. Cell health is critical to maximal performance. Cell viability should be ≥ 90% at time of transfection.
- Gently invert the ExpiFectamine™ Sf Transfection Reagent 5–10 times before use to ensure thorough mixing.
- Undiluted bacmid DNA is added directly to pre-diluted ExpiFectamine™ Sf Transfection Reagent in Opti-MEM™ I Reduced Serum Medium. See Appendix A, "Troubleshooting" for additional information.
- Complexation of bacmid DNA and ExpiFectamine™ Sf Transfection Reagent takes place at room temperature for 5 minutes using cold (4°C) or room temperature reagents.

  Note: Longer complexation times (over 20 minutes) may lead to loss in performance and are not recommended.
- For maximal flexibility, the ExpiSf™ Expression System allows you to rapidly generate high-titer baculovirus using a simple suspension-based transfection protocol. Transfection of bacmid DNA using this method results in a high-quality, high-titer P0 baculovirus stock that can be used directly in protein expression experiments.
- Transfection of suspension ExpiSf9™ cells can be done in 24 deep-well plate or 125-mL shake flask format. See Table 2 for scaling up and down transfections. After cell incubation for 3–5 days post-transfection, a high-titer P0 virus can be obtained.

Note: If you would like to generate baculovirus using the standard adherent-based transfection method, see Appendix E, "Transfect ExpiSf9™ cells using adherent-based transfection protocol" for detailed instructions.

Transfect ExpiSf9™ cells

During all cell manipulations, mix the cells by gentle swirling; avoid vigorous mixing/pipetting. Cell health is critical to maximal performance. See Table 3 for suggested volumes for transfection at various scales.

1. Subculture and expand ExpiSf9™ cells until the cells reach a density of approximately 5×10^6– 10×10^6 viable cells/mL and ≥90% viability.

2. At the day of transfection, determine viable cell density and percent viability.

   Note: The cells should have reached a density of approximately 5×10^6–10×10^6 viable cells/mL. Viability should be ≥ 90% to proceed with transfection.

3. Dilute cells to 2.5×10^6 cells/mL in 25 mL ExpiSf™ CD Medium in a 125-mL non-baffled, vented shake flask.

   Use the following steps to prepare the cells:

   a. Pipet 62.5×10^6 viable cells into a sterile 50-mL conical tube.

   b. Centrifuge at 300 × *g* for 5 minutes.

   c. Aspirate the supernatant and gently resuspend cells in 25 mL fresh ExpiSf™ CD Medium.

   d. Transfer the cell suspension to a 125-mL shake flask.

4. Allow cells to recover for 0–30 minutes in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator on an orbital shake platform set at 125 ±5 rpm (for shakers with a 19-mm or 25-mm shaking diameter) or 95 ±5 rpm (for shakers with a 50-mm shaking diameter).

5. Prepare ExpiFectamine™ Sf/bacmid DNA complexes using cold (4°C), or room temperature reagents as described.

   Note: Do not keep reagents on ice during complexation. Simply remove reagents from refrigeration and commence with DNA complexation.

   a. Gently invert the ExpiFectamine™ Sf Transfection Reagent 5–10 times to mix.

   b. Dilute ExpiFectamine™ Sf Transfection Reagent with Opti-MEM™ I Reduced Serum Medium, then mix by inverting the tube 5–10 times.

   c. Incubate diluted ExpiFectamine™ Sf Transfection Reagent for 5 minutes at room temperature.

   d. Add bacmid DNA to the diluted ExpiFectamine™ Sf Transfection Reagent. Mix by gently inverting the tube 5–10 times.

      Note: It is not necessary to pre-dilute bacmid DNA prior to addition.

   e. Incubate ExpiFectamine™ Sf /bacmid DNA for 5 minutes at room temperature.

6. Slowly transfer the mixture dropwise to the prepared shake flask from Step 4.

7. Incubate the cells in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator on an orbital shaker platform set at 125 ±5 rpm (for shakers with a 19-mm or 25-mm shaking diameter) or 95 ±5 rpm (for shakers with a 50-mm shaking diameter) for 72–96 hours.

Baculovirus generation: Isolate and amplify P0 viral stock

General guidelines

- After transfecting ExpiSf9™ cells, budded virus should be released into the medium 72–96 hours after transfection. Once the cells appear infected (i.e., demonstrate characteristics typical of late to very late infection), harvest the virus from the cell culture medium.
- Harvest virus when cells have dropped to 60–80% viability (typically 72–96 hours post-transfection).

  Note: In some instances it may take up to 120 hours for enough virus to be generated.

  Note: Optimal harvest time may vary depending on the specific properties of the baculovirus being expressed. See "Baculovirus generation" on page 26.
- Use ExpiSf9™ cells that are in excellent health, low passage (5–20), log phase growth, and ≥ 90% viability.
- Use a low MOI (0.05 – 0.1) to infect ExpiSf9™ cells. Higher MOI will reduce baculovirus titer (See Appendix F, "Multiplicity of Infection" for information on how to calculate MOI).
- You cannot amplify the baculovirus indefinitely, as the viral genome sequence will acquire deleterious mutations with each passage.
- Viral stocks produced using the ExpiSf™ Expression System are stable at 4°C for up to 12 weeks. For long-term storage, store an aliquot of the viral stock at −80°C or lower for later use.
- Do not store routinely-used viral stock at temperatures below 4°C. Repeated freeze/thaw cycles can result in a 10– to 100–fold decrease in virus titer.

Required materials not supplied

- Transfected ExpiSf9™ cells
- Hemocytometer or an automated cell counter
- Trypan Blue Solution, 0.4%
- Orbital shaker in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator (See Table 2 for recommended shaker speeds)

Isolate P0 virus

1. After transfected cells show signs of late stage infection (typically 72–96 hours post-transfection), collect the virus-containing medium from the shake flask, then transfer to sterile conical tubes.

   Note: The optimal virus harvest time can vary from 72–120 hours post-transfection. See Appendix A, "Troubleshooting"

2. Centrifuge tubes at 300 × *g* for 5 minutes to remove cells and large debris.

3. Transfer the clarified supernatant to fresh conical tubes.

   This is the P0 viral stock. Store at 4ºC, protected from light.

4. After you have obtained clarified P0 viral stock, you may:
   a. Use the P0 viral stock to infect ExpiSf9™ cells in protein expression experiments.

b. Determine the titer of your viral stock (see Appendix D, "Baculovirus titering assay").

Note: The typical range of P0 virus titer at Day 4 suspension of ExpiSf9™ cells in ExpiSf™ CD Medium following the recommended protocol is: 1×10^9–5×10^9 infectious viral particles (ivp)/mL.

c. If required, amplify the viral stock to generate larger volumes of your virus (See next section).

Guidelines to amplify baculovirus stock

- If necessary, follow the procedure to amplify your P0 viral stock using suspension ExpiSf9™ culture in 1-L shake flask format.
- Suspension-based transfection of ExpiSf9™ cells allows you to generate a high-titer P0 virus stock that can be used directly in protein expression experiments. No virus amplification is needed.

Day –1: Prepare cells

1. On the day before infection, seed ExpiSf9™ cells at 1×10^6 viable cells/mL in 200 mL total culture volume in ExpiSf™ CD Medium using a 1-L non-baffled, vented shake flask.
2. Incubate cells overnight in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator on an orbital shaker platform set at 125 ±5 rpm (for shakers with a 19-mm or 25-mm shaking diameter) or 95 ±5 rpm (for shakers with a 50-mm shaking diameter).

Day 0: Infect cells

1. On the next day, determine viable cell density and percent viability.
   Viable cell density should be approximately 1×10^6–2.5×10^6 viable cells/mL and ≥90% viability.
2. Infect cells using 100–200 µL of 1:10 dilute P0 viral stock.

   Note: If P0 virus stock titer was determined, calculate the volume of virus stock required to infect cells with an MOI of 0.05–0.1.
3. Incubate infected cells in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator on an orbital shaker platform set at 125 ±5 rpm (for shakers with a 19-mm or 25-mm shaking diameter) or 95 ±5 rpm (for shakers with a 50-mm shaking diameter).

Day 3–5: Harvest virus

1. When cell viability drops to 60–80%, collect virus-containing medium and transfer to sterile conical tubes.
2. Centrifuge tubes at 300 × *g* for 5 minutes to remove cells and large debris.
3. Transfer the clarified supernatant to fresh conical tubes.
   This is the P1 viral stock. Store at 4°C, protected from light.

Protein expression in ExpiSf9™ cells

General guidelines

- After you have generated a viral stock with an appropriate titer (≥1 × 10^8 ivp/mL), you can use this viral stock to infect ExpiSf9™ cells and assay for expression of your recombinant protein.
- Allow freshly thawed cells to recover in culture for two or more passages post-thaw before infecting.
- During all cell manipulations, mix the cells by gentle swirling and avoid vigorous mixing.
- The ExpiSf™ Expression System allows for high-density infection of ExpiSf9™ cells in suspension.
- The ExpiSf™ Enhancer is added to the cell suspension at the time of cell seeding, 18–24 hours before infection. This component is essential for high infection efficiency and achieving superior protein yields.

  Note: ExpiSf9™ cells are infected 18–24 hours after ExpiSf™ Enhancer.
- At the time of cell infection, viable cell density should be between 5 × 10^6–7 × 10^6 cells/mL.
- Titering the baculovirus stock before infecting ExpiSf9™ cells is highly recommended as this ensures optimal infection kinetics and consistent conditions across multiple expression runs. An MOI of 5 is recommended with the ExpiSf™ Expression System.
- If you have generated a high-titer baculovirus stock from the pFastBac™ 1-Gus Control Vector, use this recombinant baculovirus in your experiments as an expression control.

Required materials not supplied

- ExpiSf9™ cells in ExpiSf™ CD Medium at 5 × 10^6–10 × 10^6 viable cells/mL and ≥ 90% viability
- High-titer P0 or P1 baculovirus stock for your protein of interest
- Hemocytometer or an automated cell counter
- Trypan Blue Solution, 0.4%
- Orbital shaker in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator (See Table 2 for recommended shaker speeds)

Protein expression at various scales

Table 4 Recommended volumes for protein expression using the ExpiSf™ Expression System at different scales. Numbers provided are on a per-shake flask or per-well basis.

Vessel type	24 deep-well plate	125-mL	250-mL	500-mL	1-L	2-L
Total number of cells	20×10^6 cells	1.25×10^8 cells	2.5×10^8 cells	5×10^8 cells	10×10^8 cells	2×10^9 cells
Final viable cell density	5×10^6 cells/mL					
Initial culture volume	4 mL	25–30 mL	50–60 mL	100–120 mL	200–240 mL	400–480 mL
ExpiSf™ Enhancer	16 µL	100 µL	200 µL	400 µL	800 µL	1.6 mL
Volume of P0 baculovirus stock[1]	40–80 µL	250–500 µL	500–1000 µL	1–2 mL	2–4 mL	4–6 mL
MOI (if virus titer was determined)[2]	5					
Shaker speed	250 rpm (19-mm shaking diameter)	125 ±5 rpm (19-mm shaking diameter) 125 ±5 rpm (25-mm shaking diameter) 95 ±5 rpm (50-mm shaking diameter)				
Recommended flask type	—	PETG, sterile, non-baffled, vented shake flask				

[1] Recommended volume of baculovirus stock. In some instances, this volume will need to be empirically determined.

[2] Recommended starting MOI. In some instances, optimal MOI will need to be empirically determined. See Appendix A, "Troubleshooting" for further recommendations.

Infect ExpiSf9™ cells

Subculture, then expand ExpiSf9™ cells until the cells reach a density of approximately 5×10^6–10×10^6 viable cells/mL and ≥90% viability.

Day –1: Prepare ExpiSf9™ cells for infection

1. On the day before infection, determine viable cell density and percent viability.
   The cells should have reached a density of approximately 5×10^6–10×10^6 viable cells/mL. Viability should be ≥ 90% to proceed with infection.
2. Seed the cells from Step 2 to a final density of 5×10^6 viable cells/mL with fresh ExpiSf™ CD Medium, pre-warmed to room temperature.
   Gently swirl the flask to mix the cells.
3. Immediately after seeding, add ExpiSf™ Enhancer to the shake flask.
4. Incubate cells overnight in a 27°C non-humidified, air-regulated, non-CO_2 atmosphere incubator on an orbital shaker platform set at 125 ±5 rpm (for shakers with a 19-mm or 25-mm shaking diameter) or 95 ±5 rpm (for shakers with a 50-mm shaking diameter).

Day 0: Infect ExpiSf9™ cells

1. 18–24 hours after addition of ExpiSf™ Enhancer, determine viable cell density and viability.

   Cell density should be approximately 5×10^6–7×10^6 viable cells/mL and ≥80% viability.

2. Infect the cells using high-titer (≥1×10^8 ivp/mL) virus stock at an MOI of 5.

   If virus titer was not determined, add 250–500 µL of high-titer virus stock to the shake flask.

3. Incubate cells in a 27°C non-humidified, air-regulated, non-CO_2 atmosphere incubator on an orbital shaker platform set at 125 ±5 rpm (for shakers with a 19-mm or 25-mm shaking diameter) or 95 ±5 rpm (for shakers with a 50-mm shaking diameter).

4. Harvest cells (or media, if the recombinant protein is secreted) at the appropriate time (e.g., 48–72 hours post-infection).

   Note: Optimal time to harvest protein depends on the specific properties of the protein being expressed and the protocol selected. Typical harvest times can range from 24–120 hours post-infection.

Optimize protein expression

Expression levels vary depending on the specific recombinant protein expressed. However, the ExpiSf™ Expression System exhibits consistent expression level for any particular protein from one infection to the next.

When expressing a protein for the first time, use the following parameters to optimize the titer of your recombinant protein.

- **MOI**: Infect cells at varying MOIs (e.g., 1, 2, 5, and 10) and assay for protein expression.
- **Virus volume**: Infect cells using varying volumes of baculovirus (e.g., 100 µL, 200 µL, 500 µL, and 1 mL) and assay for protein expression.
- **Time course**: Infect cells at a constant MOI or volume and assay for recombinant protein expression at different time points post-infection (e.g., 24, 48, 72, and 96 hours post-infection).

Troubleshooting

General cell culture

Observation	Possible cause	Recommended action
Cells are not recovering after resuscitation	Improper cell thawing	Expect cell viability to be ≥80% 3 days after resuscitation. In the first 1–2 passages, the ExpiSf9™ cells grow with a doubling time of approximately 24 hours when following the subculture guidelines in this protocol. If cells are not reaching ≥80% viability after 3 days, we recommend thawing a new vial.
	Improper storage cells	Immediately on receipt of the cells on dry ice, either thaw the cells immediately or place the vials into liquid nitrogen storage for at least 72 hours to allow cells to acclimate before resuscitation. For long-term storage, store cells in liquid nitrogen, vapor phase. Do not store the cells at –80°C.
Cells are growing slowly and have longer than expected doubling times	Improper cell resuscitation and/or handling	ExpiSf9™ is a high-density cell line, therefore subculture the cells when cell density are at log phase growth between 5×10^6–10×10^6 viable cells/mL. Subculturing cells outside of this growth can negatively affect growth and cell performance.
		During cell manipulations, mix the cells by gentle swirling and avoid vigorous mixing/pipetting, especially immediately before transfection and infection.
	Improper subculturing routine	To maintain a healthy culture, grow ExpiSf9™ cells to a density of 5–10×10^6 cells/mL to ensure that the cells have reached log phase growth, then subculture the cells to a density of 0.5×10^6 cells/mL or 1.0×10^6 cells/mL, for a 4 or 3 day subculturing scheme, respectively.
		If the cells significantly overgrow above 10×10^6 cells/mL, subculture the cells to a density of 0.5×10^6 cells/mL to allow the cells to recover.

Observation	Possible cause	Recommended action
Cells are growing slowly and have longer than expected doubling times	Improper shaking speed	As shaking speed is critical to optimal cell growth, use the following shaking speeds for shakers with different orbital diameters. • 125 ±5 rpm for shakers with 19-mm shaking diameter • 125 ±5 rpm for shakers with 25-mm shaking diameter • 95 ±5 rpm for shakers with 50-mm shaking diameter
	Improper incubator temperature	Ensure that the equipment is calibrated for temperature. Do not allow the incubator temperature to exceed 28°C. The total heat from the incubator and the shaker can cause cell culture temperatures to exceed the recommended range and lead to decreased cell growth, clumping, or cell death. In such instances, reduce the temperature setting of the incubator to compensate for heat generated by the shaker.
Cells grow well, then die after some time in culture	Suboptimal cell culture conditions	Ensure that your equipment, shaking speed, incubator temperature, flask size, and culture volumes are appropriate for your culture setup. See "Subculture ExpiSf9™ cells" on page 13 and adjust the parameters accordingly.
Cells are clumping during maintenance cell culture	High cell density	Minimal cell clumping can be observed as cells reach higher cell densities. In such instances, allow the clumps to settle and take cells from the supernatant. Do not attempt to break up clumps.
Cells have overgrown the suggested 10×10^6 cells/mL	Too many cells	If cells significantly overgrow 10×10^6 viable cells/mL during routine subculturing, passage the cells down to 0.5×10^6–1.0×10^6 viable cells/mL during the next passaging to reduce stress on the cells. Subculture cells a couple of times and monitor cell viability and growth kinetics to ensure that the cells are recovered and healthy (i.e., ≥90% viability and reaching a viable cell density of $\geq 5 \times 10^6$ cells/mL 3–4 days post-passaging) before proceeding with experiments. **Note:** If cells are allowed to overgrow above 10×10^6 viable cells/mL on multiple occasions and growth patterns have been affected, start a new, healthier ExpiSf9™ culture.

Baculovirus generation

Observation	Possible cause	Recommended action
Low virus yield	Poor quality bacmid DNA	Ensure pure bacmid DNA (with no empty bacmid) is used. Consider screening other DH10Bac™ transformants (e.g., other white colonies). Check the quality of the recombinant bacmid DNA by agarose gel electrophoresis to ensure bacmid integrity.
	Suboptimal cell growth conditions	Cell health at the time of transfection is critical to maximal performance. Ensure that cells are in the recommended density range during routine subculturing, and at the time of transfection. During cell manipulation, swirl flasks to resuspend the cells and do not shake or pipet the cells vigorously to mix, because it can lead to decreased performance.
	Cells have not recovered from resuscitation	Passage ExpiSf9™ cells at least two to three times after resuscitation and before transfection.
	Cells are plated too sparsely or too densely	Ensure cells are at the correct density at the time of transfection. With suspension ExpiSf9™ cell culture, cells are seeded at a density of 2.5×10^6 cells/mL using standard shake flasks. With adherent ExpiSf9™ cell culture,, cells are seeded at a density of 1×10^6 cells/well using a 6-well plate (see Appendix E, "Transfect ExpiSf9™ cells using adherent-based transfection protocol").
	Used too much or too little ExpiFectamine™ Sf Transfection Reagent	We have optimized the amounts of ExpiFectamine™ Sf Transfection Reagent to be used for various cultures sizes and formats. We do not recommend deviating from these recommendations as it could result in loss of transfection efficiency.
	Incubation time of lipid:DNA complexes before transfection is too long	Do not incubate diluted ExpiFectamine™ Sf Transfection Reagent for longer than 5 minutes before adding bacmid DNA because it can lead to reduced transfection efficiency.
		Add lipid:bacmid DNA complexes to cells, 5 minutes post-complexation, by drop-wise addition to the flasks with swirling. Do not allow complexation reaction to incubate for longer than 20 minutes as this will reduce transfection efficiency.
	Low transfection efficiency	Use ExpiFectamine™ Sf Transfection Reagent for transfection.

Observation	Possible cause	Recommended action
Low virus yield		Harvest baculovirus supernatant when signs of infection are visible (typically, >72–96 hours post-transfection). In some cases, it may take up to 120 hours for enough virus to accumulate. Isolate recombinant virus at different time points post-transfection to determine the optimal virus harvest time.
		During a typical transfection in suspension format, the following cell characteristics are observed: • Viability decreases to 60–80% after 4 days. • Viable cell density is 4×10^6–8×10^6 after 4 days. • Cell diameter increases to 18–20 µm after 4 days.

Protein expression

Observation	Possible cause	Recommended action
Low protein yield	Poor quality baculovirus	Ensure that a single white colony is picked during bacmid preparation to avoid a mixture of recombinant and non-recombinant bacmids. We recommend using the suspension protocol for high-quality virus, as multiple amplifications following the classical adherent format can result in spontaneous excision of the gene of interest, and formation of defective virus particles.
	Virus stock contains a mixture of recombinant and non-recombinant baculovirus	Ensure that a single white colony is picked during bacmid preparation to avoid a mixture of recombinant and non-recombinant baculovirus.
	Baculovirus does not contain the gene of interest recombinant	Ensure transposition by PCR analysis of bacmid DNA using the pUC/M13 Forward and Reverse primers.
		Transfect ExpiSf9™ cells with a new recombinant bacmid DNA prep.
	Improperly stored baculovirus or old baculovirus stock used	Store baculovirus stock at 4°C protected from light for up to 12 weeks. Alternatively, baculovirus stocks can be frozen and stored long term at –80°C or in liquid nitrogen (no DMSO or cryopreserve is necessary). Store frozen virus in small aliquots and keep freeze/thaw cycles to a minimum.

Observation	Possible cause	Recommended action
Low protein yield	Cell growth conditions not optimal	Cell health at the time of infection is critical for maximal performance. Ensure that cells are in the recommended density range during routine subculturing, and at the time of infection. During all cell manipulation, swirl flasks to resuspend the cells and do not shake or pipet the cells vigorously to mix, as it can lead to decreased performance.
	ExpiSf™ Enhancer not added	ExpiSf™ Enhancer is critical for obtaining high protein yields with the ExpiSf™ Expression System. Add ExpiSf™ Enhancer to ExpiSf9™ cells at the time of cell seeding (18–24 hours before virus infection). This solution can be added to the flasks without pre-warming. After adding Enhancer, return the cells to the incubator overnight. Ensure that the right amount of ExpiSf™ Enhancer was added for the expression scale of your choice.
	Incubation with ExpiSf™ Enhancer too short or too long	The total cell incubation time with ExpiSf™ Enhancer before baculovirus infection is an important aspect of the ExpiSf™ Expression System protocol. The optimal incubation time is 18–24 hours before infection. Incubating ExpiSf™ Enhancer-treated cells for longer than 28 hours can result in decreased infection efficiency and low protein titers.
	Suboptimal cell density at the time of ExpiSf™ Enhancer treatment	One day before virus infection (Day –1), seed ExpiSf9™ cells at a density of 5×10^6 cells/mL and immediately treat them with ExpiSf™ Enhancer. The next day (Day 0, 18–24 hours after ExpiSf™ Enhancer addition), ensure that the cells are at a density of $5–7 \times 10^6$ cells/mL.
	Cell density is too high or too low, at the time of virus infection	Ensure the cell density is $5 \times 10^6–7 \times 10^6$ viable cells/mL 18–24 hours after ExpiSf™ Enhancer is added. If cell density is above 8×10^6 cells/mL, then treatment with ExpiSf™ Enhancer is suboptimal and cell seeding should be repeated.
	Virus titer too low or too high	Titer the virus and optimize infection conditions by varying the MOI. If baculovirus titer was not determined, we recommend testing a series of virus stock dilutions (e.g., 1:10, 1:100, 1:1000) to determine the optimal virus stock volume to be used.
	Poor cell infection	During a typical infection, cell viability decreases to 60–80% after 3 days, viable cell density is $5 \times 10^6–7 \times 10^6$ cells/mL, and cell diameter will increase to 18–20 µm.

B Generate recombinant bacmid DNA

Overview

To generate a recombinant bacmid DNA containing your gene of interest using the Bac-to-Bac™ Baculovirus Expression System, you will clone your gene of interest into the pFastBac™ 1 Expression Vector using restriction enzyme digestion and ligation followed by transformation of the pFastBac™ recombinant construct into MAX Efficiency™ DH10Bac™ Competent Cells. For recommendations and guidelines to help you design your cloning strategy of your gene of interest into the pFastBac™ plasmid, refer to *Bac-to-Bac™ Baculovirus Expression System User Guide* (Pub No. MAN0000414).

Generate pFastBac™ construct

Required materials not supplied

See Appendix H, "Ordering information".

- TOP10 competent cells
- LB agar plate containing 100 µg/mL ampicillin
- Purified pFastBac™ 1 construct containing your gene of interest
- LB agar plates containing 50 µg/mL kanamycin, 7 µg/mL gentamicin, 10 µg/mL tetracycline, 100 µg/mL Bluo-gal, and 40 µg/mL IPTG (3 plates for each transformation and use freshly prepared plates)
- 15-mL round-bottom polypropylene tubes
- 42°C water bath
- 37°C shaking and non-shaking incubator

General guidelines

- The pFastBac™ 1 vector and its corresponding pFastBac™ 1-Gus expression control plasmid contains the ampicillin resistance gene to allow for selection in *E. coli* using ampicillin.
- The pFastBac™ 1 vector contains intact polyhedrin (PH) leader sequences.
- The pFastBac™ 1 vector is a non-fusion vector (that is, no fusion tags are present in the vector). Multiple tagged versions of the pFastBac™ 1 vector (for example, His and HBM) are available separately, see Appendix H, "Ordering information".

Cloning considerations

To ensure proper expression of your recombinant protein, your insert must contain:

- An ATG start codon for initiation of translation
- A stop codon for termination of the gene

Note: Stop codons are included in the multiple cloning site in all three reading frames.

Note: The production of recombinant proteins requires that your insert contain a translation initiation ATG. Generally, transfer vectors that contain intact polyhedrin (PH) leader sequences (for example, pFastBac™ vectors) can yield higher levels of expression than vectors that contain interrupted leader sequences. Protein translation can initiate at the mutated ATG (ATT) upstream of the multiple cloning site, however, initiation from this site is inefficient and generally does not interfere with expression and detection of recombinant protein.

Note: Refer to Appendix G, "Vector maps and multiple cloning sites"

E. coli host for transformation

After you have cloned your insert into pFastBac™ 1, transform the ligation reaction into *E. coli* and select for ampicillin-resistant transformants. You can use any recA, endA *E. coli* strain including TOP10, DH10B™, or DH5α™ for transformation. Do not transform the ligation reaction into DH10Bac™ cells.

Transformation

You may use any method of choice to transform *E. coli*. Chemical transformation is the most convenient method, while electroporation is the most efficient and method of choice for large plasmids. To select for transformants, use LB agar plates containing 100 µg/mL ampicillin.

Analyze transformants

Once you have obtained ampicillin-resistant transformants, we recommend the following:

1. Pick up to 10 transformants and culture them overnight in LB or S.O.B. containing 100 µg/mL ampicillin.
2. Isolate the plasmid DNA using your method of choice. We recommend using the PureLink™HiPure Plasmid DNA Miniprep Kit to purify high quality plasmid DNA from your *E. coli* transformants.
3. Analyze the plasmids by restriction analysis to confirm the presence and correct orientation of the insert.

   Use a restriction enzyme or a combination of enzymes that cut once in the vector and once in the insert.

Analyze transformants by PCR

You may also analyze positive transformants using PCR. Use the appropriate PCR primers and amplification conditions for your insert. If you are using this technique for the first time, you may want to perform restriction analysis in parallel. Artifacts may be obtained because of mispriming or contaminating template.

1. For each sample, aliquot 48 µL of PCR SuperMix High Fidelity into a 0.5-mL microcentrifuge tube. Add 1 µL each of the forward and reverse PCR primers.
2. Pick 10 colonies and resuspend them individually in 50 µL of the PCR SuperMix containing primers (remember to make a patch plate to preserve the colonies for further analysis).
3. Incubate reaction for 10 minutes at 94°C to lyse cells and inactivate nucleases.
4. Amplify for 20 to 30 cycles.

5. For the final extension, incubate at 72°C for 10 minutes. Store at 4°C.
6. Visualize by agarose gel electrophoresis.

Sequencing

You may sequence your construct to confirm that your gene of interest is in the correct orientation for expression. If you have cloned your gene into one of the pFastBac™-tagged vectors, verify that your gene is cloned in frame with the N-, C-, or HBM-terminal tag.

Long-term storage

Once you have identified the correct clone, be sure to purify the colony and make a glycerol stock for long-term storage. We recommend that you store a stock of plasmid DNA at –20°C.

1. Streak the original colony out for single colony on LB plates containing 100 µg/mL ampicillin.
2. Isolate a single colony and inoculate into 1–2 mL of LB containing 100 µg/mL ampicillin.
3. Grow until culture reaches stationary phase.
4. Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.
5. Store at –80°C.

Transform into DH10Bac™ competent cells

After you have generated your pFastBac™ construct, you are ready to transform purified plasmid DNA into MAX Efficiency™ DH10Bac™ Competent *E. coli* for transposition into the bacmid. You will use blue/white selection to identify colonies containing the recombinant bacmid.

Positive control

We recommend including the pUC19 DNA as a positive control for transformation and the pFastBac™ 1-Gus Control Vector (control plasmid) as a positive control for transposition as well as in your subsequent ExpiSf9™ cell transfection with bacmid DNA.

Required materials

- Your purified pFastBac™ construct (0.2 ng/µL in TE, pH 8)
- pFastBac™ 1-Gus control vector (0.2 ng/µL)
- MAX Efficiency™ DH10Bac™ Competent Cells (use 1 tube of competent cells for every transformation)
- pUC19 (supplied with the MAX Efficiency™ DH10Bac™ Competent *E. coli*; use as a control for transformation)
- LB agar plates containing kanamycin, gentamicin, tetracycline, Bluo-gal, and IPTG (Use 3 freshly prepared plates for each transformations)
- LB agar plate containing 100 µg/mL ampicillin (for plating pUC19 transformation control)
- SOC Medium
- 15-mL round-bottom polypropylene tubes

- 42°C water bath
- 37°C shaking and non-shaking incubator

Prepare LB agar plates

You will need to prepare LB agar plates containing 50 µg/mL kanamycin, 7 µg/mL gentamicin, 10 µg/mL tetracycline, 100 µg/mL Bluo-gal, and 40 µg/mL IPTG to select for DH10Bac™ transformants.

If you are preparing LB plates using a pre-mixed formulation, we recommend using Luria Broth Base instead of Lennox L. Using Lennox L plates will reduce the color intensity and may reduce the number of colonies obtained.

Note: Use Bluo-gal instead of X-gal for blue/white selection. Bluo-gal generally produces a darker blue color than X-gal.

Prepare for transformation

For each transformation, you will need one vial of competent cells and three selective plates.

- Equilibrate a water bath to 42°C.
- Warm selective plates at 37°C for 30 minutes
- Warm the SOC Medium to room temperature
- Pre-chill one 15-mL round-bottom polypropylene tube for each transformation

Transform cells

Follow the procedure below to transform MAX Efficiency™ DH10Bac™ competent cells with your pFastBac™ construct. We recommend including the pFastBac™ 1-Gus plasmid as a positive control for transposition and pUC19 DNA as a positive control for transformation in your experiment to help you evaluate your results.

1. Thaw on ice one vial of MAX Efficiency™ DH10Bac™ competent cells for each transformation.
2. For each transformation, gently mix and transfer 100 µL of the DH10Bac™ cells into a pre-chilled, 15-mL round-bottom polypropylene tube.
3. Add the appropriate amount of plasmid DNA to the cells and mix gently.
   Do not pipet up and down to mix.
   - Your pFastBac™ construct: 1 ng (5 µL)
   - pFastBac™ 1-Gus control plasmid: 1 ng
   - pUC19 DNA control: 50 pg (5 µL)
4. Incubate cells on ice for 30 minutes.
5. Heat-shock the cells for 45 seconds at 42°C without shaking.
6. Immediately transfer the tubes to ice and chill for 2 minutes.

7. Add 900 µL of room temperature SOC Medium.
   - **For pFastBac™ transformations**: Shake tubes at 37°C at 225 rpm for 4 hours.
   - **For pUC19 transformation**: Shake tube at 37°C at 225 rpm for 1 hour.
   - **For each pFastBac™ transformation**: Prepare 10-fold serial dilutions of the cells (10^{-1}, 10^{-2}, 10^{-3}) with SOC Medium . Plate 100 µL of each dilution on an LB agar plate containing 50 µg/mL kanamycin, 7 µg/mL gentamicin, 10 µg/mL tetracycline, 100 µg/mL Bluo-gal, and 40 µg/mL IPTG.
   - **For the pUC19 transformation**: Dilute the cells 1:100 with SOC Medium. Plate 100 µL of the dilution on an LB agar plate containing 100 µg/mL ampicillin.
8. Incubate plates for 24–48 hours at 37°C. Pick white colonies for analysis (see the next page for recommendations).

   Note: Monitor colonies starting 24 hours post-plating. Pick distinct white colonies that are >2 mm in diameter. It may take up to 48 hours for distinct white colonies to form.

IMPORTANT! Insertions of the mini-Tn7 into the mini-*att*Tn7 attachment site on the bacmid disrupt the expression of the LacZα peptide, so colonies containing the recombinant bacmid are white in a background of blue colonies that harbor the unaltered bacmid. Select white colonies for analysis. True white colonies tend to be large; therefore, to avoid selecting false positives, choose the largest, most isolated white colonies (>2 mm in diameter). Avoid picking colonies that appear gray or are darker in the center as they can contain a mixture of cells with empty bacmid and recombinant bacmid.

Verify the phenotype

1. Pick up to 10 white colonies and restreak them on fresh LB agar plates containing 50 µg/mL kanamycin, 7 µg/mL gentamicin, 10 µg/mL tetracycline, 100 µg/mL Bluo-gal, and 40 µg/mL IPTG. Incubate the plates overnight at 37°C.
2. From a single colony confirmed to have a white phenotype on restreaked plates containing Bluo-gal and IPTG, inoculate a liquid culture containing 50 µg/mL kanamycin, 7 µg/mL gentamicin, and 10 µg/mL tetracycline.
3. Isolate recombinant bacmid DNA using the procedure provided on the next page for analysis.
4. Analyze the recombinant bacmid DNA to verify successful transposition to the bacmid. We recommend using PCR to analyze your bacmid DNA.

   It is possible to verify successful transposition to the bacmid by using agarose gel electrophoresis to look for the presence of high molecular weight DNA. This method is less reliable than performing PCR analysis as high molecular weight DNA can be difficult to visualize.

Isolate and analyze recombinant bacmid DNA

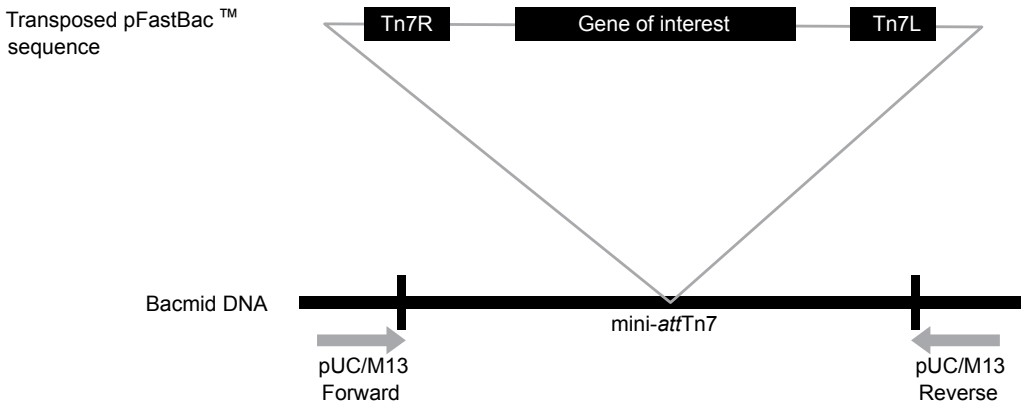
Isolate recombinant bacmid DNA

We recommend using the PureLink™ HiPure Plasmid Purification Kits to purify your bacmid DNA, which allow you to purify high-quality bacmid DNA from DH10Bac™ *E. coli*. The midi/maxi size kits allow you to have sufficient bacmid DNA to use in large-scale suspension-based transfections. For detailed instructions to purify your bacmid DNA using PureLink™ HiPure Plasmid Prep Kits. Follow instructions in *PureLink™ HiPure Plasmid Purification Kits User Guide*, (Pub. No. MAN0000486) using the MaxiPrep low-copy plasmid instructions. See Appendix H, "Ordering information").

Note: We do not recommend the PureLink™ HiPure Precipitator Module or the PureLink™ HiPure Plasmid Filter Midi/Maxiprep Kits for isolating bacmid DNA.

Analyze recombinant bacmid DNA by PCR

Recombinant bacmid DNA is greater than 135 kb in size. We recommend using PCR analysis to verify the presence of your gene of interest in the recombinant bacmid. Use the pUC/M13 Forward and Reverse primers that hybridize to sites flanking the mini-attTn7 site within the *lacZα*-complementation region to facilitate PCR analysis.



PCR analysis with pUC/M13 primers

To verify the presence of your gene of interest in the recombinant bacmid using PCR, you may:

- Use the pUC/M13 Forward and Reverse primers
- Use a combination of the pUC/M13 Forward or Reverse primer and a primer that hybridizes within your insert.

Note: The pUC/M13 Forward and Reverse primers must be custom synthesized.

Primer	Sequence
pUC/M13 Forward	5′-5′-CCCAGTCACGACGTTGTAAAACG-3′-3′
pUC/M13 Reverse	5′-5′-AGCGGATAACAATTTCACACAGG-3′-3′

DNA polymerase

You may use any DNA polymerase of your choice for PCR including Platinum™ *Taq* DNA Polymerase. If the expected PCR product is > 4 kb, we recommend using a polymerase mixture such as Platinum™ *Taq* High Fidelity DNA Polymerase for best results.

Produce the PCR product

Follow this procedure to amplify your recombinant bacmid DNA using the pUC/M13 Forward and Reverse primers and Platinum™ *Taq* DNA Polymerase. If you are using a combination of the pUC/M13 Forward or Reverse primers and a gene-specific primer, you will need to determine the amplification conditions to use. If you are using another polymerase, follow the manufacturer's recommendations for the polymerase you are using.

Amplification conditions may need to be optimized if your insert is > 4 kb.

1. For each sample, set up the 50 µL PCR reaction in a 0.5-mL microcentrifuge tube according to the following table.

Component	Amount
Recombinant bacmid DNA (100 ng)	1 µL
10X PCR Buffer (appropriate for enzyme)	5 µL
10 mM dNTP Mix	1 µL
50 mM $MgCl_2$	1.5 µL
PCR Primers (1.25 µL each 10 µM stock)	2.5 µL
Sterile water	38.5 µL
Platinum™ *Taq* DNA Polymerase (5 units/µL)	0.5 µL
Total Volume	**50 µL**

2. Amplify using the cycling parameters according to the following table.

Step	Time	Temperature	Cycles
Initial Denaturation	3 minutes	93°C	1X
Denaturation	45 seconds	94°C	25–35X
Annealing	45 seconds	55°C	
Extension	5 minutes	72°C	
Final Extension	7 minutes	72°C	1X

3. Remove 5–10 µL from the reaction and analyze by agarose gel electrophoresis.

PCR product size

Successful transposition using the pUC/M13 Forward and Reverse primers for amplification, gives PCR product sizes on agarose gel according to the following table.

Sample	PCR product size
Bacmid alone	~300 bp
Bacmid transposed with pFastBac™ 1	~2300 bp + size of your insert
Bacmid transposed with pFastBac™ 1-Gus	~4200

If you have used a combination of the pUC/M13 Forward or Reverse primer and a gene-specific primer for amplification, you will need to determine the expected size of your PCR product. Refer to the table to help you calculate the expected size of your PCR product.

C

Assay for ß-glucuronidase

Assay for ß-glucuronidase

The assay for β-glucuronidase (Gus) expression is based on a fluorogenic β-glucuronidase substrate that produces a fluorescent signal when cleaved by the Gus enzyme. This signal can be measured using a standard fluorescent plate reader. Other methods are also suitable.

Required materials not supplied

See Appendix H, "Ordering information"

- Gus-expressing baculovirus-infected ExpiSf9™ cells
- Recombinant *E. coli* Beta Glucuronidase (Gus) protein
- Fluorescein Di-β-D-Glucopyranoside (FDGlu) Substrate
- 50% Dimethyl Sulfoxide (DMSO) solution in water (v/v %)
- 96-well non-treated polypropylene microplates
- Fluorescence microplate reader (e.g. Varioskan™ LUX by Thermo Fisher Scientific)
- Halt™ Protease Inhibitor Cocktail, EDTA-free (100X)

Procedure

Step 1: Prepare Gus test sample

1. 72 hours after infecting ExpiSf9™ cells with Gus-expressing baculovirus, remove shake flasks from the incubator and transfer 300 µL of the cell suspension to a microcentrifuge tube.
2. Centrifuge samples at 300 × *g* for 5 minutes.
3. Separate pellet and supernatant and analyze separately.
4. Resuspend the cell pellet in 300 µL PBS by pipetting up and down, then add 3 µL Halt™ Protease Inhibitor Cocktail (100X) to each resuspended pellet.
5. Lyse the cells by performing 3 freeze-thaw cycles following the steps (other cell lysis methods may also be used):
   a. Transfer samples to a −80°C freezer, then incubate for 2–3 minutes.
   b. Quickly transfer samples to a 37°C water bath, then incubate for 2–3 minutes.
   c. Remove samples from the 37°C water bath and vortex for 10 seconds.

d. Repeat Steps a–c two additional times.

6. Centrifuge samples at 16,000 × *g* for 10 minutes and transfer the supernatant to fresh microcentrifuge tubes.

   This is the Gus test sample. Keep sample on ice.

Step 2: Dilute Gus test sample

1. Using microcentrifuge tubes, prepare five serial dilutions of your Gus test sample according to the following table.

Sample	Dilution	Gus test sample volume	PBS volume	Total volume per well
Dilution 1	1:100	5 µL undiluted test sample	495 µL	500 µL
Dilution 2	1:2	250 µL Dilution 1	250 µL	500 µL
Dilution 3	1:2	250 µL Dilution 2	250 µL	500 µL
Dilution 4	1:2	250 µL Dilution 3	250 µL	500 µL
Dilution 5	1:2	250 µL Dilution 4	250 µL	500 µL

2. Transfer 40 µL of each Gus test sample dilution (Dilutions 1–5) to duplicate wells of a 96-well black assay plate (that is, 2 wells for each dilution of the Gus test sample).

Step 3: Prepare GUS recombinant protein standard

1. Using microcentrifuge tubes, prepare four 2-fold serial dilutions of the Gus recombinant protein standard. See Appendix H, "Ordering information"; (concentration: 250 Activity Units/mL) according to the following table.

Sample	Dilution	Gus standard volume	PBS volume	Total volume per well	Final activity units/mL
Dilution 1	1:2	250 µL undiluted Gus standard	250 µL	500 µL	125
Dilution 2	1:2	250 µL Dilution 1	250 µL	500 µL	75
Dilution 3	1:2	250 µL Dilution 2	250 µL	500 µL	37.5
Dilution 4	1:2	250 µL Dilution 3	250 µL	500 µL	18.8

2. Transfer 40 µL of undiluted Gus recombinant protein standard and 40 µL of each dilution (Dilutions 1–4) to duplicate wells of a 96-well black assay plate (that is, 2 wells for each dilution of the Gus standard).

Step 4: Prepare FDGlu Substrate

1. Reconstitute the lyophilized FDGlu Substrate in 1 mL 50% DMSO solution (in water; v/v %). Mix by gently pipetting up and down.
2. Dilute the entire volume (1 mL) of reconstituted FDGlu Substrate in 9 mL PBS in an opaque bottle to protect from light. This is the FDGlu Substrate stock solution (concentration: 730 µM).
3. Prepare a FDGlu Substrate working solution by performing a 7.3-fold dilution of the FDGlu Substrate stock solution, from Step 2 (working solution final concentration 100 µM).
4. Calculate the total volume of working solution required to analyze your test sample; you will need 60 µL of FDGlu Substrate working solution per Gus test sample (Step 2) and Gus standard (Step 3).
   For example: For 20 samples (10 Gus standard Dilutions , 8 Gus test sample Dilutions and 2 Substrate Background Controls), prepare a total of 1.32 mL FDGlu Substrate working solution (+ 10%) by diluting 180 µL FDGlu Substrate stock solution in 1.14 mL PBS.

Step 5: Set up assay plate

1. Add 60 µL FDGlu Substrate working solution prepared in step 4 to each well containing Gus test sample and Gus standard, from Steps 2 and 3, respectively. Note: At this point, total assay volume per well should be 100 µL.
2. Add 60 µL FDGlu Substrate working solution and 40 µL PBS to two wells (these are your Substrate Background Control wells). Total assay volume should be 100 µL.
3. Add 100 µL PBS to two wells (these are your plate Background Control wells).
4. Cover the assay plate with an adhesive plate sealer and mix by gently tapping the sides of the plate.
5. Incubate assay plate at 37°C for 10 minutes without shaking.
6. Measure fluorescence on a microplate reader at 490 nm Excitation and 514 nm Emission settings.

Step 6: Calcuate Gus protein concentration in test sample

1. Prepare a standard curve by plotting the average relative fluorescence units (RFU) of each Gus standard dilution well versus its Gus Activity Units.

   Note: The Gus Activity Units in each Standard dilution is provided in Step 3. Gus Recombinant Protein standard preparation
2. Obtain the best fit curve for your data.
3. Choose a Gus test sample dilution well with an RFU value that falls within the linear range of the standard curve.
4. Use the RFU value and the best fit linear curve equation to calculate Gus Activity (Units/mL) in your Gus Test Sample by solving for x.
5. The typical yield of Gus measured after 72 hours post-infection is approximately 250,000 Activity Units/mL in the cell pellet fraction.

D Baculovirus titering assay

Baculovirus titering assay

This flow cytometric titering assay is based on the detection of the baculovirus envelope protein gp64 displayed on the surface of infected cells. Briefly, ExpiSf9™ cells are plated in a 24-well plate and 10-fold dilutions of the test virus stock are used to infect cells for 14–16 hours. Virus-infected cells are then measured by flow cytometry based on gp64 expression. This method allows faster titer determination compared to other titration methods such as plaque assay and $TCID_{50}$.

Required materials not supplied

See Appendix H, "Ordering information".

- Attune™ NxT Acoustic Focusing Cytometer (Red UV Laser configuration excitation: 633–647 nm; Emission: 660 nm)
- Baculovirus Envelope gp64 Monoclonal Antibody (AcV1), APC, (eBioscience™ (0.125 mL at 0.2 mg/mL)
- PBS solution containing 2% Fetal Bovine Serum (FBS)
- 24-well suspension plate

General guidelines

This protocol takes place over two days. Plates should be set up in the afternoon on Day 1 and harvested in the morning on Day 2, for a total of 14 to 16 hours incubation time.

Titer baculovirus: Day 1

1. Use microcentrifuge tubes to prepare five 10-fold serial dilutions (dilution range: 1×10^{-1} to 1×10^{-5}) of the test virus stock in fresh ExpiSf™ CD Medium according to the following table.

   Note: To prevent virus carryover and inaccurate titers, it is imperative to change pipet tips and vortex tubes briefly between each serial dilution.

Sample	Dilution	Virus stock volume	Diluting medium volume[1]	Total volume
Dilution 1	1:10	120 µL undiluted virus stock	1.08 mL	1.2 mL
Dilution 2	1:100	120 µL Dilution 1	1.08 mL	1.2 mL
Dilution 3	1:1000	120 µL Dilution 2	1.08 mL	1.2 mL
Dilution 4	1:10,000	120 µL Dilution 3	1.08 mL	1.2 mL
Dilution 5	1:100,000	120 µL Dilution 4	1.08 mL	1.2 mL

[1] Use fresh ExpiSf™ CD Medium to dilute virus samples.

2. In a conical tube, dilute ExpiSf9™ cells to 1.25×10^6 viable cells/mL in ExpiSf™ CD Medium.

3. Set up reactions in a 24-well suspension plate as follows:

   1. Add 1 mL of Dilutions 2, 3, 4, and 5 (from Step 1) to appropriate wells (1 well per Dilution).

      Note: You will not use Dilution 1.

   2. Add 1 mL fresh ExpiSf™ CD Medium to a negative control (i.e., no virus) well.
   3. Add 800 µL of cells at 1.25×10^6 viable cells/mL (from Step 2) to wells containing Dilutions 2, 3, 4, and 5 as well as the negative control well.

      Note: Avoid using outer plate wells to prevent evaporation. Fill any unused well with 1 mL ExpiSf™ CD Medium or PBS.

4. Incubate plate overnight at 27°C in a non-humidified incubator on a shaking platform (19-mm diameter) set to 225 ±5 rpm.

Titer baculovirus: Day 2 (A.M.)

1. Prepare the following reagents:
   - **Dilution Buffer**: PBS containing 2% FBS. You will need approximately 1.1 mL of buffer per sample.
   - **Anti-Baculovirus Envelope gp64 APC antibody**: Dilute antibody in Dilution Buffer to a final concentration of 0.15 µg/mL. You will need 100 µL diluted antibody (0.015 µg) per sample (0.75 µL antibody stock per mL).

2. Prepare samples for analysis by flow cytometry:
   a. After 14–16 hours of incubation, remove the 24-well suspension plate from the incubator and transfer the contents of each well into separate FACS tubes. Use one FACS tube per well.

   b. Centrifuge tubes at 300 x g for 5 minutes. Carefully aspirate and discard supernatants.

   c. Resuspend each cell pellet in 100 µL diluted antibody. Mix by briefly vortexing for 3–5 seconds.

   d. Incubate tubes at room temperature for 30 minutes.

   e. Wash samples by adding 1 mL PBS followed by centrifugation at 300 x g for 10 minutes.

      Carefully aspirate and discard supernatants.

   f. Resuspend each cell pellet in 1 mL Dilution Buffer.

3. Analyze samples on a flow cytometer (Red Laser - Excitation: 633–647 nm; Emission: 660 nm). Record percent positive gp64-expressing cells for viral dilution and negative control samples. Refer to the instrument manual for setup of acquisition parameters.

4. Determine virus titer:
   a. Choose the dilution sample (from Step 1, Day 1) that yields percent gp64-positive cells of < 10%.

   b. Using the cell number per well (i.e., 1 x 10^6 cells/mL), the optimal dilution sample (from Step I above), and the percent gp64-positive cells in that respective dilution sample, calculate viral titer using the equation below.

$$Viral\ Titer\ \left(\frac{ivp}{mL}\right) = \left(\frac{Cell\ number\ x\ Percent\ gp64\ positive\ cells}{Dilution\ of\ virus\ stock}\right)\ x\ 0.01$$

Example: If the percent gp64-positive cells is 6.91% when virus stock is diluted to 1 x 10^{-3} using 1 x 10^6 cells/well, the titer of the virus stock is calculated as follows:

$$Viral\ Titer\ \left(\frac{ivp}{mL}\right) = \left(\frac{1E+06\ x\ 6.91}{0.001}\right)\ x\ 0.01 = 6.91E+07\ ivp/ml$$

E

Transfect ExpiSf9™ cells using adherent-based transfection protocol

Adherent-based transfection protocol

General guidelines

- During all cell manipulations, mix the cells by gentle swirling; avoid vigorous mixing/pipetting. Cell health is critical to maximal performance.
- Subculture and expand ExpiSf9™ cells until the cells reach a density of approximately 5×10^6– 10×10^6 viable cells/mL and ≥ 90% viability.

Transfect cells

1. At the day of transfection, determine viable cell density and percent viability.

   The cells should have reached a density of approximately 5×10^6– 10×10^6 viable cells/mL. Viability should be ≥ 90% to proceed with transfection.

2. Seed the cells from Step 1 to a final density of 1×10^6 viable cells/well in a 6-well plate with a total culture volume of 3 mL in ExpiSf™ CD Medium per well.

3. Allow cells to attach for 30–60 minutes in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator.

4. Prepare ExpiFectamine™ Sf/bacmid DNA complexes using cold (4°C) or room temperature reagents, as described.

   Note: It is not necessary to keep reagents on ice during complexation. Simply remove reagents from refrigeration and commence with DNA complexation.

   a. Gently invert the ExpiFectamine™ Sf Transfection Reagent bottle 5–10 times to mix.

   b. Dilute 10 µL ExpiFectamine™ Sf Transfection Reagent with 250 µL Opti-MEM™ I Reduced Serum Medium. Mix by inverting the tube 5–10 times.

   c. Incubate diluted ExpiFectamine™ Sf Transfection Reagent for 5 minutes at room temperature.

   d. Add 1 µg bacmid DNA to the diluted ExpiFectamine™ Sf Transfection Reagent. Mix by gently inverting the tube 5–10 times.

      Note: It is not necessary to pre-dilute bacmid DNA prior to addition.

   e. Incubate ExpiFectamine™ Sf /bacmid DNA for 5 minutes at room temperature.

5. Slowly transfer the mixture dropwise to the 6-well plate from Step 4.

6. Incubate the cells in a 27°C non-humidified, air regulated non-CO_2 atmosphere incubator on a stationary platform for 72–120 hours.

F Multiplicity of Infection

Multiplicity of Infection calculation

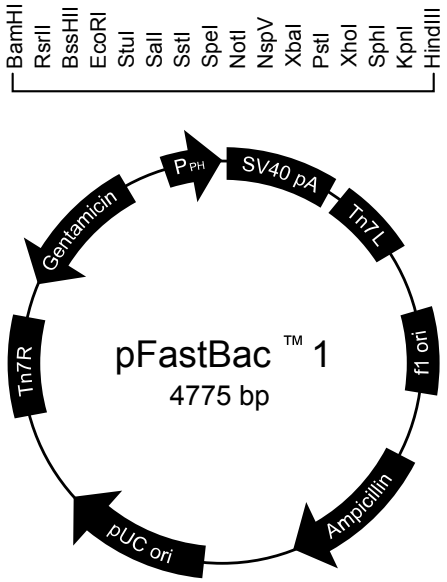
Multiplicity of Infection (MOI) is defined as the number of virus particles per cell. Use the following formula to calculate how much viral stock (inoculum) to add to obtain a specific MOI:

$$Amount\ of\ virus\ required = \frac{MOI\ x\ number\ of\ cells}{titer\ of\ virus\ stock\ \left(\frac{ivp}{ml}\right)}$$

G Vector maps and multiple cloning sites

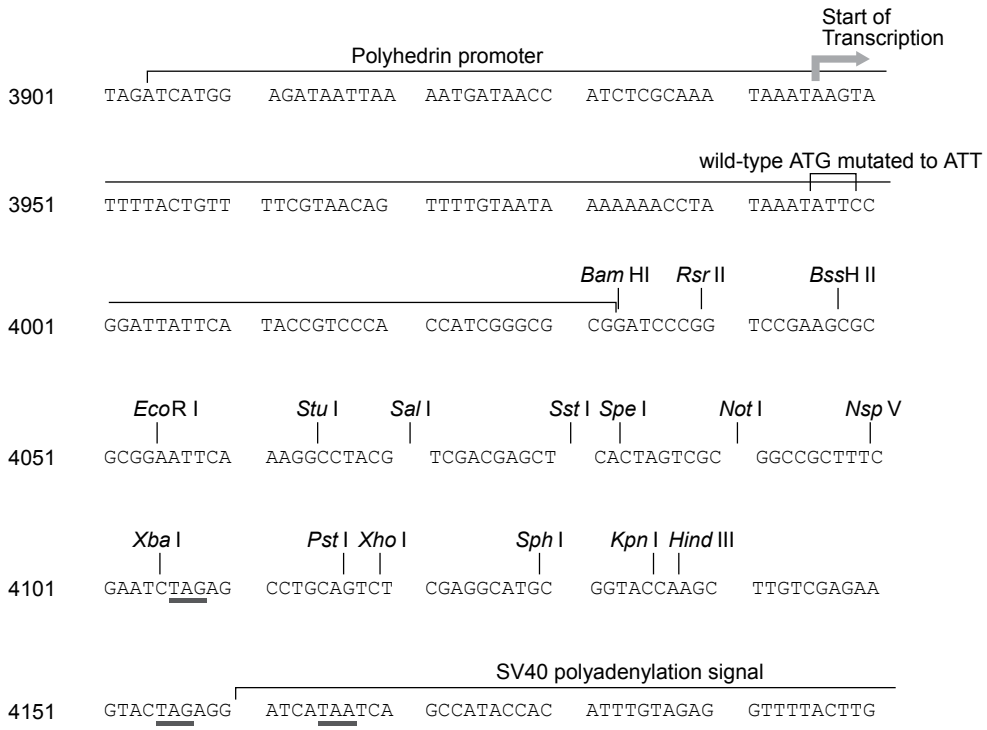
pFastBac™ 1 vector map

The vector sequence of pFastBac™ 1 is available from **thermofisher.com** or by contacting Technical Support.



- f1 origin: bases 2–457
- Ampicillin resistence gene: bases 589–1449
- pUC origin: bases 1594–2267
- Tn7R: bases 2511–2735
- Gentamicin resistence gene: bases 2802–3335 (complementary strand)
- Polyhedrin promoter (P_{PH}): bases 3904–4032
- Multiple cloning site: bases 4037–4142
- SV40 polyadenylation signal: bases 4160–4400
- Tn7L: bases 4429–4594

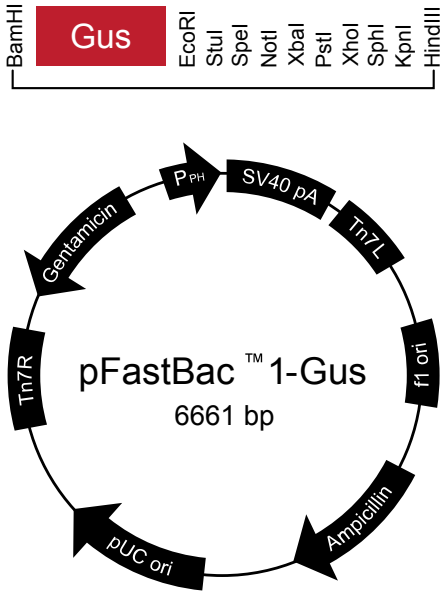
Multiple cloning site in pFastBac™ 1 vector



Restriction sites are labeled to indicate the actual cleavage site. Potential stop codons are underlined. The vector sequence of pFastBac™ 1 is available from **thermofisher.com** or by contacting Technical Support.

pFastBac™ 1-Gus vector map

The vector sequence of pFastBac™ 1-Gus is available from **thermofisher.com** or by contacting Technical Support.



- f1 origin: bases 2–457
- Ampicillin resistence gene: bases 589–1449
- pUC origin: bases 1594–2267
- Tn7R: bases 2511–2735
- Gentamicin resistence gene: bases 2802–3335 (complementary strand)
- Polyhedrin promoter (P_{PH}): bases 3904–4032
- GUS ORF: bases 4081–5892
- SV40 polyadenylation signal: bases 6047–6287
- Tn7L: bases 6315–6480

Ordering information

Unless otherwise indicated, all materials are available through **thermofisher.com**.

ExpiSf™ Expression System

Item	Amount	Source
ExpiSf™ Expression System Starter Kit		
ExpiSf™ Expression System Starter Kit	1 kit	A38841[1] A39112[2] A39111[3]
ExpiSf™ Expression System Starter Kit with SmartStart Training	1 kit + training	TRN00503
ExpiSf™ Expression System components		
ExpiSf9™ Cells (1 × 10^7 cells/mL; 1.5 mL)	1 vial	A35243
ExpiFectamine™ Sf Transfection Reagent	1 mL	A38915
ExpiSf™ CD Medium	500 mL 1000 mL 6 × 1000 mL 10 L Bag 20 L Bag	A3767801 A3767802 A3767803 A3767804 A3767805
ExpiSf™ Protein Production Kit	For 1 L of culture For 10 L of culture For 50 L of culture	A3767806 A3767807 A3767808
Bac-to-Bac™ Vector Kit	1 kit	10360-014
MAX Efficiency™ DH10Bac™ Competent Cells	5 × 100 µL	10361012
Opti-MEM™ I Reduced Serum Medium	100 mL	31985062
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile	24 × 125 mL	4115-0125

[1] North America, Europe
[2] Asia Pacific, Japna, Latin America
[3] China

Bac-to-Bac™ and related products

Item	Amount	Source
pFastBac™ HT Vector Kit	1 kit	10584-027
pFastBac™ Dual Expression Vector	10 µg	10712-024
Bac-to-Bac™ Baculovirus Expression System	1 kit	10359-016
Bac-to-Bac™ C-His TOPO™ Expression System	1 kit	A11100
Bac-to-Bac™ N-His TOPO™ Expression System	1 kit	A11101
Bac-to-Bac™ HBM TOPO™ Secreted Expression System	1 kit	A11339
Bac-to-Bac™ C-His TOPO™ Cloning Kit	1 kit	A11098
Bac-to-Bac™ N-His TOPO™ Cloning Kit	1 kit	A11099
Bac-to-Bac™ HBM TOPO™ Cloning Kit	1 kit	A11338
Fluorescein Di-ß-D-Glucopyranoside (FDGlu) Substrate	5 mg	F2881
Baculovirus Envelope gp64 Monoclonal Antibody (AcV1), APC (0.2 mg/mL)	25 µg	17-6991-80
Recombinant *E. coli* Beta Glucuronidase (GUS) protein	25,000 units	abcam - ab58415

Cloning products

Item	Amount	Source
One Shot™ TOP10 Chemically Competent *E. coli*	20 reactions	C4040-03
Ampicillin, sodium salt, irradiated	200 mg	11593-027
Kanamycin Sulfate	100 mL	15160-054
Gentamicin (50 mg/mL)	10 mL	15750060
Bluo-Gal	1 g	15519-028
Isopropylthio-ß-galactoside (IPTG)	1 g	15529-019
Platinum™ PCR SuperMix High Fidelity	100 reactions	12532016

Plasmid purification products

Item	Amount	Source
PureLink™ HiPure Plasmid Midiprep Kit	1 kit	K210004
PureLink™ HiPure Plasmid Maxiprep Kit	1 kit	K210006
PureLink™ HiPure Expi Plasmid Megaprep Kit	1 kit	K210008XP
PureLink™ HiPure Expi Plasmid Gigaprep Kit	1 kit	K210009XP

Protein extraction and quantification products

Item	Amount	Source
I-PER Insect Cell Protein Extraction Reagent	250 mL	89802
Pierce™ Protease Inhibitor XL Capsules, EDTA-free	10 capsules	A37989
Halt™ Protease Inhibitor Cocktail, EDTA-free (100X)	5 mL	78437
Pierce™ Protease Inhibitor Tablets, EDTA-free	20 tablets	A32965
Pierce™ Rapid Gold BCA Protein Assay Kit	250 mL	A53226

Protein purification products

Item	Amount	Source
HisPur™ Ni-NTA Resin	100 mL	88222
Pierce™ Ni-NTA Magnetic Agarose Beads	5 mL	78606
HisPur™ Ni-NTA Superflow Agarose	250 mL	25215
Pierce™ Glutathione Superflow Agarose	10 mL	25236
Pierce™ Anti-DYKDDDDK Magnetic Agarose	1 mL	A36797
Pierce™ Anti-DYKDDDDK Affinity Resin	1 mL settled	A36801
Pierce™ Anti-HA Agarose	1 mL	26181
POROS™ CapSure™ AAVX Affinity Resin	10 mL	A36739
POROS™ PI 50 Weak Anion Exchange Resin	50 mL–10 L	1-2459
POROS™ XQ Strong Anion Exchange Resin	10 mL–10 L	82073
POROS™ HS 50 Strong Cation Exchange Resin	50 mL–10 L	1-3359

Nalgene™ flasks products

Item	Amount	Source
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile	24 × 125 mL	4115-0125
	12 × 250 mL	4115-0250
	12 × 500 mL	4115-0500
	6 × 1000 mL	4115-1000
	4 × 2000 mL	4115-2000
	4 × 2800 mL	4115-2800
Thomson Instrument Company 24 deep-well plate with lid	Case of 20	NC0012954[1]
Greiner Bio-One™ 96-well non-treated polypropylene microplates	Case of 100	07-000-110[1]
Greiner Bio-One™ CELLSTAR™ 24-well plate	Case of 100	07-000-670[1]

[1] fisherscientific.com

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf